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TITLE: Analysis of Small Intestinal Microbiome in Children with Autism

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14. ABSTRACT
The goal of this project is to analyze the entire microbial population of the mucosa in the upper gastrointestinal tract of children with autism to determine if there is an overgrowth of specific populations of bacteria and analyze the relationship between the intestinal flora and behavior. Our current sub-contractor from the institute of Genome Sciences, University of Maryland School of Medicine was not able to amplify and sequence DNA from the duodenal biopsies of autistic children and controls. A new sub-contractor – the Research and Testing Laboratory was found. A new set of duodenal biopsies from children with and without autism will be sent to the Research and Testing Laboratory for DNA extraction, PCR for 16S rRNA, sequencing. No additional funding will be requested to complete this project.

15. SUBJECT TERMS- Autism, duodenal microbiome, DNA extraction, PCR, sequencing

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INTRODUCTION

The overall goal of the project is to analyze the entire microbial population of the mucosa in the upper gastrointestinal (GI) tract of children with autism to determine if there is an overgrowth of specific populations of bacteria and analyze the relationship between the intestinal flora and behavior. In aim 1, we will analyze duodenal microbiome of children with and without ASD who have had a biopsy taken for evaluation of GI symptoms. In aim 2, the results of aim 1 will be correlated with questionnaires on GI symptoms and autistic behavior. The duodenal biopsies were collected by the Digestive Function Laboratory personnel and preserved at -80° C in tissue repository. Microbiome studies will include DNA isolation from duodenal mucosa of 20 children with autism and 20 controls, the 16S rRNA amplification with PCR, and shotgun reads on a 454 Life Sciences pyrosequencer followed by the computational analysis.

BODY

In this study, we have to analyze duodenal microbiome in children with autism. According to the original proposal the mucosal biopsies from children with and without autism were sent to the sub-contactor Dr. Jacques Ravel's laboratory (Institute for Genome Sciences, University of Maryland School of Medicine) for processing. DNA was extracted from all biopsies but the laboratory was not able to amplify the microbial DNA. In the e-mail from 8/14/12 Dr. Ravel wrote that "the sequences we got were not good at all" and "it really doesn't look promising that we will get anything useful out of the DNA we extracted with the MolZym Kit". To make sure that DNA extracted with MolZyme kit can not be sequenced, we asked to send DNA extracts to the Research and Testing Laboratory (Lubbock, TX). Unfortunately people in this laboratory also were not successful with PCR and DNA sequencing from the samples extracted in Dr. Ravel's lab. The problem appears to be the extraction process in Dr. Ravel's lab.

We have sent over 700 intestinal mucosal biopsy samples to the Research and Testing Laboratory before and people in the laboratory have successfully sequenced the samples when they isolated DNA using their proprietary technique.

We were determined to study the intestinal microbiome in the duodenum of children with autism and the only way to do this is to take new biopsies which we currently have in our biorepository and send them to the Research and Testing Laboratory.

We contacted the Research and Testing Laboratory and they agree to be our new sub-contractor. We also asked DOD to change the sub-contractor and give us a no-cost extension. Approval from the DOD to change the sub-contractor was received on 8/24/2012 and no-cost extension was issued on 12/03/12. A new Agreement, as well as SOW and SOI, was prepared for the Research and Testing Laboratory and sent out on 12/27/12. According to these documents, new biopsies from 20 autistic and 20 non-autistic children will be taken from our biorepository and send to the Research and Testing Laboratory for DNA extraction, PCR, and 16S rRNA sequencing.

We have the funding remaining from the original budget and will not require any additional funding to complete this project.

KEY RESEARCH ACCOMPLISHMENTS

A new sub-contractor was found. Duodenal biopsies from 20 autistic children and 20 children with normal development were selected from the Digestive Function Laboratory biorepository and are ready to be send to the Research and Testing Laboratory for analysis.

REPORTABLE OUTCOMES

No outcomes to report at the present time

CONCLUSION

The sub-contractor from the Institute for Genome Sciences, University of Maryland School of Medicine was not able to amplify and sequence DNA from the duodenal biopsies of autistic children and controls. A new sub-contractor was chosen based on its performance. A new set of duodenal biopsies will be send to the Research and Testing Laboratory for DNA extraction, PCR, and sequencing. No additional funding will be requested to complete this project.